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Research Article

Virtual Screening of Putative Anticonvulsant Hydantoin Derived Drugs and Biological Evaluation

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Abstract

It has been found that derivatives of benzyl *N*-piperidin-4-one have similar effect than phenytoin drug as an anticonvulsant for the treatment of epilepsy according to docking simulation studies. This is due to the similar binding mode in these chemicals. In this study, a series of six *N*-piperidinespirohydantoins derivatives were prepared by Bucherer-Bergs reaction, from corresponding *N*-piperidin-4-ones derivatives benzyl and phenethyl groups, substituted in the aromatic ring with metoxy and nitro groups. The hydantoins were evaluated for anticonvulsant activity with maximal electroshock (MES) induced method *in vivo* in male Wistar rats in the treatment of epilepsy.

ABBREVIATIONS

MES: Maximal Electro Shock; AEDs: Anti Epileptic Drugs; CASH: Cycloalkanespirohydantoins; ADT: Autodock Tools; PSH: Piperidinespirohydantoins; TLC: Thin-Layer Chromatography; IR: Infrared Spectra; TMS: Tetramethylene Silane; MS: Mass Spectra; ESI-MS: Electrospray Ionization Mass Spectra; LC/MSD: Liquid Chromatography/ Mass Spectra Detector

INTRODUCTION

The search for new chemical compounds that have biological activity, efficiency, effectiveness, reproducibility, stability, and security, has become paramount to the constant improvements on current medication. This is important because of a trend in patients of developing resistance to outdated and sometimes even current medication creating a constant need for more innovative pharmaceutical products.

Neurodegenerative diseases have been a huge challenge for the development of new drugs, since many of them are not attractive from a commercial point of view. This is the case with epilepsy, which is one of many diseases that require lifelong treatment for the patient to remain in a controlled manner and to be allowed to do all their independent normal activities [1].

Epilepsy is the most common neurological disorder throughout the world. It is characterized by current unpredictable epileptic seizures, muscle spasms, physical injury, damage to the brain, and unconsciousness, to name a few symptoms that manifest within this disease. As thus, there exists a need for the development of new anti seizure medication with improved efficacy and tolerability, as several of the currently available antiepileptic drugs (AEDs) have been associated with undesirable side effects [2-4].

There are several drugs that are able to control this disease; one of the most used is the phenytoin, which contains a hydantoin ring. Hydantoin (imidazolidine-2,4-dione) moiety constitutes an attractive and multifaceted pharmacological scaffold present in several drugs [7]. This small and rigid heterocyclic backbone, has potential to act on various pharmacological targets.

Hydantoin-1,3-imidazolidinedione derivatives [5,6] Figure (1) exhibit diverse and interesting pharmacological properties [7,8]. Several such derivatives (phenytoin, mephenythoin, norantoin, methetoin, ethotoin, osphenytoin) are renowned anticonvulsive drugs [9,10] (Table 1).

The anticonvulsant activity of hydantoins has been known since 1938 when Merrit and Putman discovered that 5,5-diphenylhydantoin (phenytoin) Figure (2) showed anti-epileptic acivity [11]. Phenytoin acts on brain by reducing electrical conductance among neurons by stabilizing the inactive state of voltage gated sodium channel [12-14] (Table 2).

Table 1: Yield of the alkylated piperidin-4-ones **6a-f.**

Entry	n	R	*N*-Piperidinespirohydantoins (yield %)[a]
6a	1	2-NO_2	96
6b	1	3-NO_2	98
6c	1	4-NO_2	98
6d	1	3-OCH_3	83
6e	1	4-OCH_3	86
6f	2	4-OCH_3	70

n: number of methylene
R: type and position of the groups in the aromatic system

Table 2: Yield of the Bucherer-Bergs reaction to produce the substituted piperidinspirohydantoins **7a-f.**

Entry	n	R	*N*-Piperidinspirohydantoins (yield %)[a]
7a	1	2-NO_2	73
7b	1	3-NO_2	72
7c	1	4-NO_2	71
7d	1	3-OCH_3	76
7e	1	4-OCH_3	73
7f	2	4-OCH_3	70

n: number of methylene
R: type and position of the groups in the aromatic system

Other5-substitutedhydantoinssuchas5,5-dithienylhydantoin, 5,5-dipyridylhydantoin, spirothio-hydantoin, thiohydantoin and dithiohydantoins also possess anticonvulsive activity [13]. Hydantoin derivatives can also be found in antiarrhythmic (azimilide), antimicrobial agents (nitrofurantoin), skeletal muscle relaxants (dantrolene) and nonsteroidal antiandrogens (nilutamide), while allantoin is used as a keratolytic, astringent, antiacid and antipsoriatic drug [15]. Hydantoins also display antidepressant, antiviral and antithrombotic activities, as well as inhibitory activity against some enzymes (human aldose reductase and human leucocyte elastase) [16]. Finally, some herbicides (spirohydantoin, thioxohydantocidin), fungicides (clodantoin) and insecticides also have the hydantoin skeleton in their structure [17,18].

Voltage-gated sodium channels are the molecular targets for several important commonly used classes of drugs including anticonvulsant drugs (antiepileptic), local anesthetic and antiarrhythmic drugs, and antidepressants, which bind in the inner pore of the channel with affinities related to the channel gating states. In fact, all of these types of drugs block sodium currents with noticeable voltage dependence, showing low affinity at resting states and strong block for open states [19,20].

The Na+ channel is a multimolecular complex (Figure 1) [21]. A large α-subunit forms the channel's pore and gating machinery and contains the principal drug binding sites. This α-subunit is composed of four highly homologous domains (I–IV), each with six trans-membrane α-helical segments (S1–S6) assembled in a clockwise pattern around the central pore. The pore itself has a fairly large outer vestibule, formed by the four extracellular P loop segments between each S5 and S6.

These P loops fold back into the membrane to make a shallow funnel, with the innermost four residues (the DEKA motif) forming the selectivity filter. The channel's activation gate is located near the inner mouth of the pore, and it includes hydrophobic residues from the four S6 segments (Figure 1).

Anticonvulsant drugs typically have a tricyclic structure, with a hydantoin ring in the middle, like **2**. Cycloalkanespirohydantoins (**CASH**, Figure 2,3 a-f) and piperidinespirohydantoins (**PSH**, Figure 2,4 a-c) are two new classes of molecules that are designed to be potential anticonvulsant drugs candidates; they also contain a hydantoin ring and were recently synthesized from cycloalkanones and N-piperidin-4-ones under microwave-assisted conditions [22] (Figure 2).

The interaction of this set of new molecules with the open state of the neuronal Na+ channel was explored by means of docking simulations to the aim of investigating their putative action as anticonvulsant drugs on the basis of their binding mode and their binding affinity for the channel. Insight into the structural basis of their block of neuronal Na+ channels may help to explain their target specificity and assist in a rational search for new anticonvulsants. On these bases, some derivatives of a very favored compound, were then synthesized and tested *in vivo* for their anticonvulsant activity.

The benzene dimer was the prototypical system to study the π stacking, stabilization energy is 2-3 kcal. This interaction π-π is favored by the stacking of aromatic systems, it is weak, but

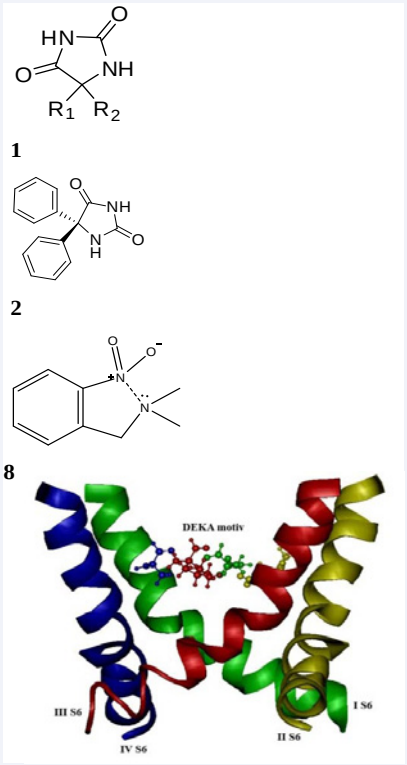


Figure 1 Na+ open channel model (Lipkind and Fozzard, 2005) used for the docking simulations, coloured by chain. The S6 helix of each domain is labeled. In ball and stick are the four DEKA residue of the selectivity filter [21].

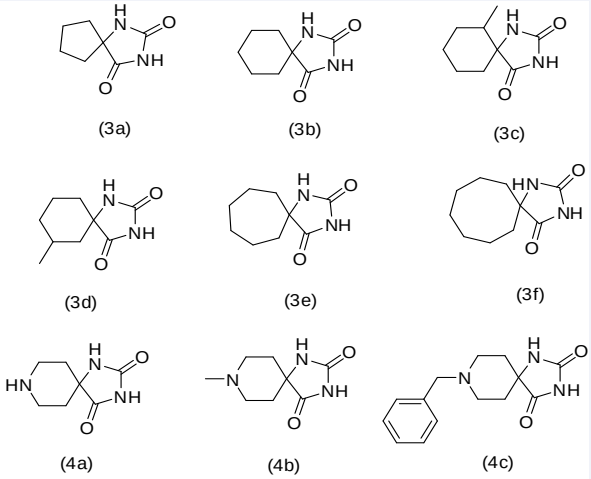


Figure 2 Cycloalkanespirohydantoins (CASH, 3a-f) and piperidinespiro hydantoins (PSH, 4a-c).

very important to increase the affinity between complex-drug-candidate. Aromatic systems should have availability of electron density to increase the induced quadrupole moment between aromatic systems. Noncovalent interactions between the side chains of the aromatic amino acids phenylalanine (Phe), tyrosine (Tyr), histidine (His), and tryptophan (Trp) amino acids were examined by Rappe et al., [23].

MATERIALS AND METHODS

Docking simulations methods

The Na+ channel structure was obtained from the supplementary materials of the work of Lipkind and Fozzard [21]. They developed a model of the activated, open channel pore based on the structure of the open MthK channel. As previously state, the open, activated channel creates the high-affinity binding site for these sodium channel putative blocker drugs. The used structure contains the domains' region forming the inner open pore of the Na+ channel, namely the residue 71-96 of the S6 helix of each domain and the residues forming the DEKA selectivity ring.

The receptor structure was prepared by means of the Autodock Tools (ADT) software package [24], adding hydrogens and assigning Kollman partial charges set to the atoms [25].

The ligands, namely **CASH 3 a-f** and **PSH 4 a-c**, were built by means of Hyperchem8 [26] software package and then prepared for the docking simulations with ADT, assigning Gasteiger partial charges set [27].

The Autodock Vina [28], software was used for docking simulations. The grid maps had a box size of 18 x 21.8 x 17.2 Å, and was centered in the mouth of the channel pore.

Structural analysis of the binding modes was made using ADT, VMD [29,30], software and the web tool for macromolecular visualization First Glance in Jmol [31].

General procedures

Thin-layer chromatography (TLC) was performed on silica gel F_{254} plates (Merck). Melting points were obtained on an Electrothermal 88629 apparatus and are uncorrected. Infrared spectra (IR) were recorded on a Perkin Elmer FT-IR 1600 spectrophotometer. ^{1}H and ^{13}C nuclear magnetic resonance spectra at 200 Hz and 50.289 Hz, respectively, were recorded on a Varian Mercury 200 MHz Spectrometer in $CDCl_3$ and DMSO-d_6 with TMS as internal standard. Gas Chromatography 793 A Automatic Liquid Sampler (EM) was obtained and the intensities are reported as a percentage relative to the base peak after the corresponding *m/z* value. Electrospray ionization mass spectra (ESI-MS) were obtained with an Agilent LC/MSD ion trap.

PHARMACOLOGICAL STUDIES

Experimental animals

Male Wistar albino rats (230-270 g) provided by the Institute of Neurology and Neurosurgery in Mexico City, were used for biological testing. The Wistar rats were bred under laboratory conditions, kept in groups cages, fed with balanced meals and treated with sterile water.

Acute toxicity studies

The study was conducted as per OECD-425 guide lines for testing of chemicals acute oral toxicity. The test was used to fix the safe dose for the compounds **7a-f** and **4c**. Male Wistar albino rats were divided into ten groups each containing 10 animals and repeated for all the drugs tests. Drugs were administered by oral route in different concentrations (2000, 1000, 500, 250, 100 and 50mg/kg body weight). The animals were observed for their death over a period of 7days. The LD50 values were calculated by up and down method and dose was fixed as 50mg/kg body weight.

Maximal Electroshock test (MES)

Anticonvulsant property of the test compounds in this study was evaluated by its ability to protect against Maximal Electric Shock induced convulsions. Group 1 was the control groups which received vehicle (DMSO, PE, saline solution); Group 2 received Phenytoin (24 mg/Kg, i.p.); Groups 3-9 received one of the test compounds **7a-f** and **4c** each (24 mg/Kg, i.p.). 1 h after the administration (i.p.) of compounds, Maximal Electric Shock of 80 mA current for 1 sec was applied through auricular electrodes to induce convulsions using an Electroconvulsimeter ECT (Unit 57800 Ugo Basile) in the control, standard and test compounds treated animals. Duration of hind limb tonic extensor was noted. The percentage of protection against shock induced seizures was calculated [32].

RESULTS AND DISCUSSION

Chemistry

General procedure for the synthesis of benzyl piperidone derivatives: To a solution $CHCl_3$ (25 mL) was added 4-piperidone monohydrate (0.15 g, 0.97 mmol), and DIPEA (0.5 mL, 2.92 mmol) was added dropwise, with vigorous stirring for 30 minutes to which was subsequently added compound (**5a**) (0.42 g, 1.95 mmol). The solution was refluxed for 48 hours, after was washed with water (3x30 mL). The organic phase was extracted with ethyl acetate (3x25mL) and dried with anhydrous sodium sulfate.

The solvent was reduced under reduced pressure. Yellow solid (**6a**) was obtained which was purified using dichloromethane-petroleum ether (8:2) as mobile phase.

1-(2-nitrobenzyl) piperidin-4-one (6a). Yellow oil (0.15 g, 0.97 mmol, 96% yield). IR (ATR): 3237, 2814, 1710 cm^{-1}; RMN ^1H (200 MHz, $CDCl_3$) δ 7.84 (d, *J* = 7.6 Hz 1H), 7.60 (m, 2H, Ar-H), 7.45 (m, 1H), 3.92 (s, 2H), 2.75 (t, *J* = 5.4 Hz, 4H), 2.41 (t, *J* = 6.2 Hz, 4H), RMN ^{13}C (50 MHz, $CDCl_3$): δ 208.7, 149.8, 133.4, 132.2, 130.8, 128.3, 124.5, 58.6, 53.0, 41.2. EM (m/z): 234.

1-(3-nitrobenzyl) piperidin-4-one (6b). Yellow solid (0.15 g, 0.97 mmol, 96% yield). M.p. 82-84 °C. IR (ATR): 3064, 3026, 2924, 1493, 1453, 1742 cm^{-1}; RMN ^1H (200 MHz, $CDCl_3$): δ 8.27 (s, 1H), 8.12 (d, *J*= 7.8 Hz, 1H), 7.71(d, *J*= 7.8 Hz), 7.53 (t, *J*= 8.0 Hz), 3.73 (s, 1H,), 2.79 (d, *J*= 6.2 Hz, 4H), 2.49 (d, *J*= 6.2 Hz 4H); RMN ^{13}C (50 MHz, $CDCl_3$): δ 208.6, 140.6, 134.8, 129.4, 123.5, 122.5, 61.0, 53.0, 41.2. EM *m/z*: 234.

1-(4-nitrobenzyl) piperidin-4-one (6c): Yellow solid (0.15 g, 0.97 mmol, 98% yield). M.p. 104-106 °C. IR (ATR): 3064, 3026, 2924, 1493, 1453, 1742 cm^{-1}; RMN ^1H (200 MHz, $CDCl_3$): δ 8.19 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.56 (d, J_1= 8.8 Hz, 1H, Ar-H), 3.71 (s, 2H, N-CH_2-Ph), 2.76 (t, *J* = 6.0 Hz, 4H, $(CH_2)_2$-N), 2.47 (t, *J* = 6.2 Hz, 4H, $(CH_2)_2$-C=O-N), RMN ^{13}C (50 MHz, $CDCl_3$): δ208.6, 140.6, 134.8, 129.4, 123.5, 122.5, 61.0, 53.0, 41.2. EM *m/z*: 234.

1-(3-methoxybenzyl) piperidin-4-one (6d): Light brown oil (0.58 g, 3.83 mmol, 83 % yield). RMN ^1H (200 MHz, DMSO-d_6): δ 7.25 (dd, J_1 = 5.0, J_2 = 7.8 Hz, 1H), 6.87 (m, 3H), 3.81 (s, 2H), 3.60 (s, 3H), 2.75 (t, *J* = 6.4 Hz, 4H), 2.44 (t, *J* = 5.8 Hz, 4H); RMN ^{13}C (50 MHz, $CDCl_3$): δ 209.3, 159.7, 139.8, 129.3, 121.1, 114.4, 112.6, 61.9, 55.2, 52.9, 41.3. EM *m/z*: 234.

1-(4-methoxybenzyl) piperidin-4-one (6e): Brown oil (0.58 g, 3.83 mmol, 86% yield). RMN ^1H (200 MHz, DMSO-d_6): δ 7.26 (d, *J* = 8.8 Hz, 2H, Ar-H), 6.87 (d, *J* = 8.8 Hz, 2H), 3.80 (s, 3H), 3.56 (s, 2H), 2.73 (t, *J* = 6.0 Hz, 4H), 2.44 (t, *J* = 6.2 Hz, 4H); RMN ^{13}C (50 MHz, $CDCl_3$): δ 209.4, 158.9, 130.1, 113.7, 61.3, 55.2, 52.7, 41.3. EM *m/z:* 234.

1-(4-methoxyphenethyl) piperidin-4-one (6f): Yellow oil, (0.14 g, 3.25 mmol, 72% yield), IR (ATR): 2796, 2, 1702, 1510 cm^{-1}; RMN ^1H (200 MHz, $CDCl_3$): δ 7.16 (d, *J* = 8.6 Hz, 2H), 6.84 (d, *J* = 8.6 Hz, 2H), 3.79 (s, 3H), 2.80 (m, 6H), 2.72 (m, 2H), 2.47 (t, *J* = 6.2, Hz, 2H); RMN ^{13}C (50 MHz, $CDCl_3$): δ 209.0, 158.0, 132.0,129.6, 113.8, 59.5, 55.2, 53.0, 41.2, 33.2. EM *m/z*: 233.

General procedure for the synthesis of piperidinspiro-hydantoins from N-alkyl-piperidin-4-ones: To a solution of methanol-water (1:1) (10mL) was added compound **6a-f** (0.24 g, 1.02 mmol), KCN (0.14 g, 2.15 mmol), and $(NH_4)_2CO_3$ (0.39g, 4.08 mmol). The mixture reaction was stirred at 55 °C for 12 hours. After the reaction time, ethyl ether (20mL) was added to precipitate the product which was washed with water (3x30mL) and ethyl ether (3x30mL). A solid white compound was obtained **7a-f** without further purification.

8-(2-nitrobenzyl)-1,3,8-triazaspiro[4.5]decane-2,4-dione (7a): White solid (0.833 g, 4.00 mmol, 73 % yield). M.p. 207-209 °C. IR (ATR): 3223, 2918, 1765, 1709 cm^{-1}. ^1H NMR (200 MHz, DMSO-d_6) δ 7.80 (d, *J* = 7.6 Hz, 1H), 7.85-7.45 (d, *J* = 15.6 Hz, 1H), 7.70-7.36 (m, 9H). ^{13}C NMR (50 MHz, DMSO-d_6) δ 178.2, 156.4, 149.7, 133.0, 132.7, 131.1, 128.6, 124.2, 60.1, 58.2, 48.3, 33.2. EM *m/z*: 304.

8-(3-nitrobenzyl)-1,3,8-triazaspiro[4.5]decane-2,4-dione (7b): White solid (0.755 g, 6.79 mmol, 72 % yield). M.p. 207-209 °C. IR (ATR): 3182, 2946, 1769, 1730 cm^{-1}. ^1H NMR (200 MHz, DMSO-d_6) δ 10.63 (s, 1H), 8.43 (s, 1H), 8.15-8.08 (m, 2H), 7.80-7.58 (m, 2H), 3.63 (s, 2H), 2.76-2.67 (m, 2H), 2.41-2.29 (m, 2H), 1.92-1.77 (m, 2H), 1.53 (d, *J* = 13.0 Hz, 2H). ^{13}C NMR (50 MHz, DMSO-d_6) δ 178.2, 156.4, 147.9, 141.2, 135.3, 129.8, 122.2, 122.0, 60.7, 60.1, 48.1, 33.2. EM *m/z*: 304.

8-(4-nitrobenzyl)-1,3,8-triazaspiro[4.5]decane-2,4-dione (7c): White solid (1.21 g, 4.83 mmol, 70 % yield). M.p. 232-234 °C. IR (ATR): 3220, 3179, 1773, 1712 cm^{-1}. ^1H NMR (200 MHz, DMSO-d_6) δ 10.64 (s, 1H), 8.45 (s, 1H), 8.18 (d, *J* = 8.8 Hz, 2H), 7.60 (d, *J* = 8.8 Hz, 2H), 3.63 (s, 2H), 2.73-2.77 (m, 2H), 2.39-2.28 (m, 2H), 1.94-1.79 (m, 2H), 1.53 (d, *J* = 13.2 Hz, 2H). ^{13}C NMR (50 MHz, DMSO-d_6) δ 178.1, 156.4, 147.0, 146.6, 129.6, 123.4, 61.0, 60.1, 48.2, 33.2. EM *m/z*: 304.

8-(3-methoxybenzyl)-1,3,8-triazaspiro[4.5]decane-2,4-dione (7d): White solid (0.76 g, 3.01 mmol, 76 % yield). M.p. 219-221 °C. IR (ATR): 3055, 2932, 1653, 1598, 1254 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 10.64 (s, 1H), 8.44 (s, 1H), 7.22 (t, *J* = 7.8 Hz, 1H), 6.88-6.78 (m, 3H), 3.73 (s, 3H), 2.72-2.66 (m, 4H), 2.26 (t, *J* = 11.4 Hz, 2H), 1.82 (ddd, J_1 = 4.0, J_2 = 13.2, J_3 = 10.8 Hz, 2H), 1.50 (d, *J* = 12.8 Hz, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 178.3, 159.3, 156.4, 140.3, 129.3, 120.8, 113.9, 112.3, 61.9, 60.3, 55.0, 48.2, 33.2. EM *m/z*: 234.

8-(4-methoxybenzyl)-1,3,8-triazaspiro[4.5]decane-2,4-dione (7e): White solid (0.59 g, 2.50 mmol, 71 % yield). M.p. 254-256 °C. IR (ATR): 3053, 2932, 1653, 1602, 1256 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 8.45 (s, 1H), 7.20 (d, *J* = 7.6 Hz, 2H), 6.87 (d, *J* = 8.9 Hz, 2H), 2.71-2.65 (m, 4H), 2.25 (t, *J* = 9.2 Hz, 2H), 1.80 (ddd, J_1 = 3.8, J_2 = 13.0 Hz, 2H), 1.50 (d, *J* = 13.2 Hz, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 178.3, 158.3, 156.4, 130.4, 129.9, 113.6, 61.5, 60.4, 55.0, 48.1, 33.2. EM (m/z): 234.

8-(4-methoxyphenethyl)-1,3,8-triazaspiro[4.5]decane-2,4-dione (7f): White solid (1.07 g, 4.49 mmol, 70% yield). M.p. 207-209 °C. IR (ATR): 3218, 2962, 1771, 1731 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 7.09 (d, *J* = 8.6 Hz, 2H), 6.79 (d, *J* = 8.6 Hz, 2H), 3.70 (s, 3H), 2.67 (m, 4H), 2.31 (t, *J* = 9.4 Hz, 2H), 1.86 (m, 2H), 1.50 (d, *J* = 13.0 Hz, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 176.4, 155.8, 154.7, 130.6, 127.8, 111.9, 58.7, 58.3, 53.3, 46.5, 31.5, 30.3. EM *m/z*: 303.

Docking simulations

Each ligand from the two sets of putative anticonvulsants (**CASH** and **PSH**) was docked into the inner open pore of the human Na+ channel. The more energetically favored complex found for each ligand, shows in any case with the exception of **PSH 4c** a position involving an interaction of the hydrophilic rings of the molecules (the hydantoin ring and the N-cycloalkane or the piperidine rings) both with the Tyr1771 residue, or with the Phe1764 residue (human Na+ 1.2 channel residue numbering) of the S6 helix of domain IV of the channel pore (Figure 3a, 3b). Alternatively, the ring also possibly makes a sort of bridge between Phe1764 and Phe1468, which is the residue in the analogue position of Tyr1771 but in domain III (Figure 3c).

The **PSH 4c** molecule has the longer and bulkier structure; consequently, it can reach a position that only its extended length permits and that put it in contact with both the two residues reached by the other compounds (Figure 4). In fact, its aromatic ring is stacked with the aromatic ring of Tyr1771, forming a favorable aromatic-aromatic interaction, while one of the amino groups of the hydantoin ring is in position for a polar interaction with the л-electrons of the aromatic ring of Phe1764, forming an amino-aromatic interaction (Figure 3).

All **CASH** and **PSH** complexes, except **PSH 4c**, have a similar binding energy, between -5.0 and -6.0 Kcal/mol. Instead, the **PSH 4c** complex has a more favorable energy (-7.0 Kcal/mol), corresponding to a dissociation constant of 1 to 2 order of magnitude lower than other molecules, therefore a greater binding affinity. A control docking simulation was then performed on phenytoin molecule, giving a binding energy of -7.0 Kcal/mol, comparable with experimental results [32] and with the one of PSH 4c (Figure 4).

Our results are in strong agreement with the docking simulations previously performed by Lipkind and Fozzard [33] on different anticonvulsants (among which was the phenytoin); their simulations had led to similar structures for the complexes, with the drug interacting with the same two residues found by us, i. e. Phe1764 and Tyr1771. Phenytoin in particular, was oriented with one of its aromatic rings forming a non-polar aromatic-aromatic interaction with Tyr1771, while the amino groups of the hydantoin ring were forming an amino-aromatic interaction with Phe1764. The two residues were known from mutagenesis studies to be critical for the anticonvulsant drug's blocking action.

The binding energy analysis indicated the **PSH 4c** as the ligand that forms the more favored complex with Na+ channel open pore, with an affinity comparable to the phenytoin. In addition, **PSH 4c** has the same kind of interactions of

Figure 3 The three kind of ligand position (in stick, colored by element) found in the complexes of Na+ channel open pore (in cartoon, colored by chain) with CASH 3a-f and PSH 4a-b: a) near Tyr1771, b) near Phe1764 or c) bridging Phe1764 and Phe 1468. The S6 helix of each domain is labeled. In ball and stick are the four DEKA residue of the selectivity filter.

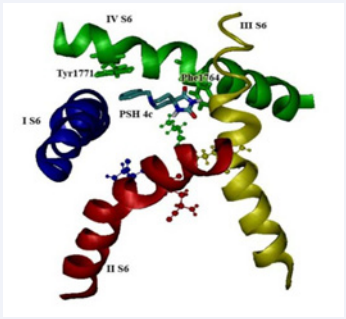


Figure 4 The best complex of the PSH 4c molecule (in stick, coloured by element) with the Na+ open channel model (in cartoon, coloured by chain). The stacking of the ligand aromatic ring with Tyr1771 is evident, as such as the amino-aromatic interaction of hydantoin ring with Phe1764. In ball and stick are the four DEKA residue of the selectivity filter.

phenytoin with the two residues Phe1764 and Tyr1771, which were experimentally shown to be important for the channel functionality. These kinds of interactions, that are also shown in other neutral anticonvulsant drugs, seem to be important for the efficacy in blocking the channel pore and the residues involved seem to be essential for channel recognition by these molecules, because they can set the orientation of the rigid drug structure into the pore [34]. In fact, the drug structure is so bulky and wide that could easily occlude the central part of the inner pore and obstruct Na+ permeation sterically. In conclusion, the **PSH 4c** molecule is the best candidate of this series of compounds to be potentially active as anticonvulsant drug.

According to the results of virtual screening, we decided to synthesize a second group of piperidinespirohydantoins derivatives, making some structural changes on **PSH 4c** molecule. We introduced a strong electron donor group (methoxy) and strong electron withdrawing (nitro) in different positions of the aromatic ring to study the effect of the substituent. The addition of these groups will be possible to determine whether electronic effects are present to differentiate the biological activity of each. Phenethyl derivative was further synthesized and substituted with a methoxy group in the para position to know the effect of the aliphatic chain. **PSH 4c** and its derivatives were then biologically tested to investigate their anticonvulsant activity *in vivo.*

The nitro groups are electron withdrawing which deactivate the aromatic system, weakening the quadrupole moment of π-π interaction. The molecules 7a, b and c, have nitro group and decreased anticonvulsant protection. In the case of 7a shows an ortho nitro effect that restricts the mobility of the aromatic system to form an N-N interaction [8]. This structural restriction does not favor π-π interaction and shows no anticonvulsant effect.

Synthesis of piperidinespirohydantoins derivatives

The alkylated piperidones were prepared from 4-*N*-piperidone monohydrate hydrochloride by a conventional alkylation reaction (Scheme 1), for obtained compounds in the range of 80-96% yield (Scheme 1).

The piperidinespirohydantoins synthesis was obtained

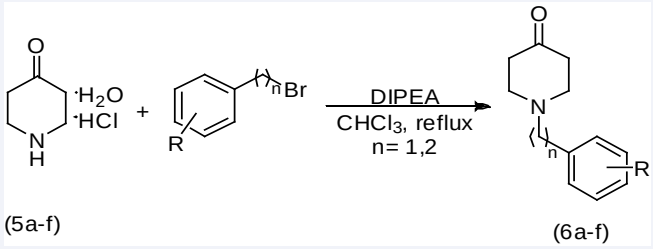
by traditional Bucherer-Bergs reaction (Scheme 2), using *N*-piperidones alkylated for obtaining piperidine-spirohydantoins derivatives in the range of 68-76% yield (Scheme 2).

The products obtained during the alkylated step were purified by column chromatography. Yields are shown in Table (1), where it is observed that benzyl derivative achieved excellent yields are achieved compared to the phenethyl derivatives. This occurs because the alkylation step for benzyl derivatives is faster, since they possess a greater number of resonance structures, unlike the phenethyl derivatives, which makes them less reactive (Table 1).

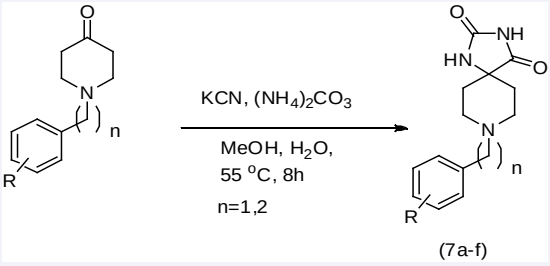
The products obtained from the classical Bucherer-Bergs reaction for the synthesis of hydantoins cores in solution, were obtained similarly in a range of 62-76% yield (Table 2). These compounds required to be washed only with ethyl ether and water for purification (Table 2).

Anticonvulsant activity

The screening of anticonvulsant activity of **PSH 4c** and its derivatives (**7a-g)** were evaluated by maximal electroshock (MES) induced seizure method after i.p administration of the drug candidate to male Wistar rats at the dose of 24 mg/Kg body mass. Seizure inducing maximal electroshock (80 mA, 1.0 sec) was applied 1 hour after the administration of drug candidate. Phenytoin was used as a control. The evaluation shown that **PSH 4c** has the best protection percentage, above phenytoin drug candidate, in the first dose. The **7e** compound displayed significant protection compared to the phenytoin. However, the compounds **7a-c**, are less active than phenytoin (Figure 5). Compound **7d** display the same percentage of protection that phenytoin dose (Figure 5).



Scheme 1 Alkylation reaction of N-piperidin-4-one with benzyl and phenethyl derivatives.



Scheme 2 Bucherer-Berg reaction for obtained piperidinspirohydantoins.

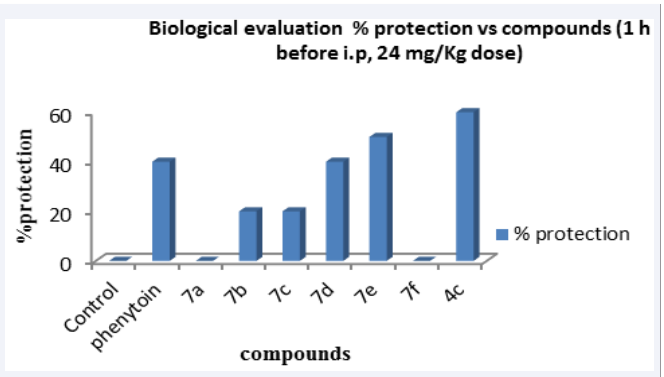


Figure 5 Graph of results of anticonvulsant activity.

CONCLUSION

The docking studies indicated the **PSH 4c** as the ligand that forms the more favored complex with Na+ channel open pore, with an affinity comparable to the one of the phenytoin. Based on these results the characteristics of the target molecule seem to be an aromatic system that interacts with Tyr1771 through a π-π stacking, and a hydrophilic moiety that interacts with Phe1764 by means of an amino-aromatic contact. Basing on these indications, hydantoins were synthesized with benzyl and phenethyl, substituted with methoxy and nitro groups to determinate the electronic effect of substituents. This study revealed that compound **4c** and **7f** shows significant anti-MES activity as predicted by the docking simulations, while its derivatives don't reach the same level of efficacy, even if in some cases have an activity higher than phenytoin. In particular, the nitro-group substituents are not very effective, and the total absence of protection that is observed when NO_2 is in the *ortho* position (compound **7a**) lead us to neglect this position for the methoxy substitutions. The methoxy group in *meta* and *para* positions (**7d** and **7e**, respectively) shows a good protection, with **7e** better than **7d**. This fact indicates that the substitution in the distal position acts better, in agreement with the absence of functionality of the *ortho* nitro-substituent. To better understand the effect of the aliphatic chain, a phenethyl derivative was studied, with a methoxy group in the *para* position (compound **7f**): the anticonvulsant activity strongly drops down to zero, suggesting that the adding of a methylene group between the two systems could prevent the aromatic stacking of the phenyl ring with Tyr1771. Therefore, further substitution on phenethyl derivatives should not be effective.

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